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Regulation of the Intracerebroventricular Administration of Brain-Derived Neurotrophic Factor on Baroreflex Function and Insulin Sensitivity in Rats

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Abstract

In addition to its well-established neurotrophic effects, brain-derived neurotrophic factor (BDNF) has also been shown to regulate glucose metabolism. The present study was conducted to determine whether BDNF has effects on baroreflex sensitivity (BRS) and whole-body insulin sensitivity through modulation of autonomic nervous function in normal rats. Male Sprague-Dawley rats were treated with intracerebroventricular BDNF (20 μg per rat, 10 μl; BDNF) or artificial cerebrospinal fluid (10 μl; control) at an infusion rate of 1 μl/min in conscious state. The whole-body insulin sensitivity was determined by the euglycemic hyperinsulinemic clamp technique. BRS in response to phenylephrine (PE-BRS) or sodium nitroprusside (NP-BRS) was assessed using linear regression analysis. The sympathetic and parasympathetic influences on BRS were investigated by pharmacological autonomic blockade. When compared to the control rats, blood glucose levels were slightly but significantly decreased in BDNF-treated rats. However, plasma insulin levels were reduced by about 30%. The whole-body insulin sensitivity was increased in BDNF-treated rats. In addition, blood pressure was increased but heart rate remained unchanged after BDNF treatment. Enhanced PE-BRS was also observed in the BDNF-treated rats, which was attributed to the abnormal parasympathetic activation as revealed by the results of the pharmacological blockade study with methylatropine. Results of the present demonstrate that central BDNF plays an important role in the regulation of whole-body insulin sensitivity and baroreflex function. The data indicate that the alteration of autonomic nervous function may play a role in the effects of BDNF.

Key Words: baroreflex, cardiac autonomic, insulin sensitivity, intracerebroventricular, neurotrophin

Introduction

Brain-derived neurotrophic factor (BDNF) plays a significant role in the development, survival and plasticity of neuronal tissues by activating the tropomyosin-related kinase receptor B (trkB) (16). BDNF and its high-affinity receptors of trkB are abundantly expressed in the adult brain, particularly

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in the hypothalamus (6, 36) and the lower brain stem (5) which functionally regulate energy balance and the autonomic nervous system. Reduction of BDNF levels in various brain regions has been implicated in the pathogenesis of neurodegenerative and psychiatric disorders (20, 22). In addition, BDNF has been reported as a neurotransmitter and/or neuromodulator (5, 14, 33, 37) in the modulation of autonomic nervous function (23). The arterial baroreflex function is predominantly dependent on both the sympathetic and parasympathetic activities. Arterial baroreceptor sensitivity (BRS) serves as an index of autonomic control of cardiovascular functions. BDNF was found to be involved in the development and survival of the arterial baroreceptor system (3, 21). However, effects of BDNF on baroreflex and cardiovascular functions are not well understood.

BDNF was also found to regulate body weight, energy expenditure, and glucose metabolism. Clinical investigations have demonstrated that impaired glucose metabolism found in type 2 diabetes is frequently associated with lower levels of BDNF (18). Studies using animal models showed that metabolic disorders, such as obesity and diabetes, could be modified by manipulation of BDNF (25, 34). Moreover, direct central effects of BDNF on glucose metabolism were also evident (28, 32). However, whether the enhancement in insulin sensitivity by central BDNF is responsible for the altered glucose metabolism remains unknown.

The present study, therefore, was designed to investigate the effects of intracerebroventricular BDNF on baroreflex function and insulin sensitivity in conscious normal rats. In addition, the roles of sympathetic and parasympathetic limbs in baroreflex control of heart rate were determined by pharmacological blockades.

Materials and Methods

Animals and Preparation

Male Sprague-Dawley rats weighing 200-250 g were purchased from the National Laboratory Animal Center (Taipei, Taiwan, ROC). The rats were housed in individual cages with a 12:12-h dark-light cycle and allowed free access to regular rat chow and tap water. All surgical procedures and experimental protocols were in compliance with the guidelines set by the Animal Care and Use Committee of Taichung Veterans General Hospital.

Surgical Procedures

Rats (n = 36) were anesthetized with chloral hydrate (400 mg/kg, i.p.; Sigma Chemical, St. Louis, MO, USA) and the head was aligned prone in a stereotaxic frame (model 1940; David Kopf Instruments, Tujunga, CA, USA). A stainless steel guide cannula (23-gauge; 17 mm) was implanted into the left lateral cerebral ventricle at the coordinates (1 mm posterior; 1.5 mm left; and 3.5 mm ventral to the Bregma skull surface) according to Paxinos and Watson (30). The guide cannula was fixed to the skull with anchoring screw and dental acrylic. A dummy cannula was placed in the guide cannula to keep the cannula shaft clear and free from debris. After cranial surgery, rats were individually housed and allowed to recover for at least a week.

Patency of the intracerebroventricular cannula was verified by the spontaneous outflow of cerebrospinal fluid and confirmed by histological verification following injection of 5 μl of Evans Blue at the end of the experiment.

Five days before experiment, the same rats additionally received vascular catheterization under chloral hydrate anesthesia as described previously (15). Femoral arterial and venous catheters were implanted for blood pressure measurements, blood sampling, and drug administration. The catheters were filled with heparinized saline (20 U/ml), exteriorized through the dorsal midscapular region of the animal, and covered with a stainless steel extension spring. Aqueous penicillin (5000 U/kg, s.c.) was administered immediately after the operation. Rats were allowed 5 days of recovery to restore preoperative body weight and activity. On the day of experiment, rats were kept undisturbed in the experimental cage for at least 1 h before experiment began. Rats were conscious and unrestrained during the experiment.

Intracerebroventricular Injection of BDNF

Intracerebroventricular injection was performed only once in the same rats, either for the assessment of autonomic control of cardiovascular function or for the euglycemic hyperinsulinemic clamp experiment. The rats were divided into two groups: control and BDNF groups (n = 18 per group). The controls received intracerebroventricular injection of artificial cerebrospinal fluid (124 mM NaC1, 2 mM KC1, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 1.25 mM KH_2PO_4, 26 mM $NaHCO_3$, 11 mM glucose), and rats in the BDNF group received intracerebroventricular injection of human recombinant BDNF (20 μg/rat, dissolved in artificial cerebrospinal fluid; PeproTech EC Ltd, London, UK). The dosage of BDNF used was previously shown to be capable of providing adequate diffusion of this molecule from the lateral ventricle to the periventricular brain tissue (13, 26, 35). BDNF or artificial cerebrospinal fluid was injected by a mic-

roinfusion pump (CMA/100, CMA/Microdialysis AB, Stockholm, Sweden) at a rate of 1 μl/min for 10 min through a 10-μl glass Hamilton syringe connected to a polyethylene tube and injection cannula. After injections, the injection cannula was kept in place for an extra minute before being withdrawn and then the dummy cannula was replaced to prevent loss of the drug or cerebrospinal fluid. The rats were allowed to equilibrate for 20 min. The effects of intracerebroventricular injection on the baseline parameters were also determined before and 20 min after injection.

Measurements of Blood Pressure, Heart Rate (HR) and Cardiac Sympathetic and Parasympathetic Influences

For measuring blood pressure and HR in the conscious rats, the arterial catheter was connected to a pressure transducer (Gould Statham P23Db, Gould Inc., Oxnard, CA, USA) and fed to a polygraph system (pressure processor and TA4000 thermal array recorder, Gould Inc.). The signals were also stored in a tape recorder (Neuro-Corder DR-890, Neuron Data, New York, NY, USA) for later analysis. The baseline measurements for mean arterial pressure (MAP) and HR were recorded 10 min before intracerebroventricular injection and were continuously monitored following intracerebroventricular injection.

Twenty min after intracerebroventricular injection, cardiac sympathetic and parasympathetic influences were evaluated based on the chronotropic effects of methylatropine bromide (4 mg/kg, i.v.; Sigma Chemical) and propranolol (5 mg/kg, i.v.; Sigma Chemical) as previously described (15). The efficacy of propranolol or methylatropine was determined by elimination of the HR responses to isoproterenol or acetylcholine by more than 95%. The parasympathetic influence was calculated by the difference between the baseline HR and the methylatropine-induced HR. The sympathetic influence was calculated by the difference between the baseline HR and the propranolol-induced HR.

Arterial BRS

BRS was determined using linear regression by plotting the reflex bradycardia or tachycardia against the moderate changes in blood pressure elicited by bolus injections of various doses of phenylephrine (PE; 0.2-10 μg/kg, i.v.; Sigma Chemical) or sodium nitroprusside (NP; 0.2-10 μg/kg, i.v.; Sigma Chemical) (15). Slopes of the regression line used as the index of BRS were calculated for each PE or NP test in each rat. The contributions of sympathetic and parasympathetic components in BRS were determined by propranolol and methylatropine blockade, respectively (15).

Euglycemic Hyperinsulinemic Clamp Experiment

The whole-body insulin sensitivity was determined by the euglycemic hyperinsulinemic clamp experiment conducted after overnight fasting as described in our previous study (15). During the clamp experiment, somatostatin (1.3 μg/kg · min; Curamed Pharma GmbH, Pforzheimer, Germany) was continuously infused to suppress endogenous insulin secretion. The insulin infusion was held at a constant rate of 4 mU/kg · min to create a steady high insulin level. The glucose infusion rate (GIR) was then adjusted to maintain the blood glucose around the euglycemic level throughout the study. The last 60 min clamp period (Clamp $_{90\text{-}150\ min}$) served as the steady-state euglycemic hyperinsulinemic period. Based on the fact that hepatic glucose production would be suppressed by circulating hyperinsulinemia (11, 31), the mean GIR at the steady-state euglycemic hyperinsulinemic period would be regarded as the whole body glucose disposal. The Si value, an index of whole-body insulin sensitivity, was defined as the mean GIR divided by the mean insulin concentration during the Clamp $_{90\text{-}150\ min}$ period. The metabolic clearance rate of insulin (MCRi) was calculated from the insulin infusion rate divided by the difference of mean insulin concentration during the Clamp $_{90\text{-}150\ min}$ and the basal period (15).

Biochemical Analysis

After overnight fast, blood samples (0.4 ml) were obtained before and 20 min after intracerebroventricular injections, and during the Clamp $_{90\text{-}150\ min}$ period. Plasma samples were divided into aliquots and frozen at -80°C for later analysis. Whole-blood glucose levels were measured by the glucose oxidase method with the YSI glucose analyzer (YSI 2300 Plus; Yellow Springs Instruments, Yellow Springs, OH, USA). Plasma insulin levels were measured by solid-phase two-site enzyme immunoassay techniques using a commercial rat insulin enzyme-linked immunosorbent assay kit (Mercodia AB, Uppsala, Sweden).

Calculation and Data Analysis

The experimental results were evaluated by two-way analysis of variance (ANOVA) with repeated measures. Bonferroni's test was applied for multiple comparisons when ANOVA detected a statistical significance in the factors. $P < 0.05$ was considered statistically significant. Values are expressed as means ± SEM.

Results

After intracerebroventricular administration of

Table 1. Baseline blood glucose, plasma insulin, blood pressure, HR, and cardiac sympathetic and parasympathetic influences before and after intracerebroventricular administration of BDNF in rats

	Control (n = 10)		BDNF (n = 10)	
	Before	After	Before	After
Blood glucose (mM)	5.3 ± 0.2	5.4 ± 0.1	5.3 ± 0.1	4.8 ± 0.1*†
Plasma insulin (pM)	162 ± 6	167 ± 7	158 ± 10	110 ± 4*†
MAP (mmHg)	111 ± 3	110 ± 4	107 ± 2	124 ± 2*†
HR (bpm)				
Baseline	344 ± 3	345 ± 4	345 ± 4	350 ± 3
AT-treated	–	392 ± 3	–	373 ± 4†
PR-treated	–	314 ± 3	–	303 ± 3†
Cardiac sympathetic influence (bpm)	–	-31 ± 2	–	-48 ± 2†
Cardiac parasympathetic influence (bpm)	–	47 ± 4	–	25 ± 4†

MAP, mean arterial blood pressure; HR, heart rate; bpm, beats/min; AT-treated, HR after methylatropine bromide treatment; PR-treated, HR after propranolol treatment; Control, rats received intracerebroventricular injection of artificial cerebrospinal fluid; BDNF, rats received intracerebroventricular injection of BDNF. *$P < 0.05$, compared to the corresponding values before intracerebroventricular injection within the same group. †$P < 0.05$ compared to the corresponding values of the control group. Values are expressed as means ± SEM.

BDNF, the blood glucose and plasma insulin levels were significantly decreased in rats, whereas there were no changes in rats after intracerebroventricular administration of artificial cerebrospinal fluid (control) (Table 1). BDNF-treated rats also showed elevation in the baseline MAP. However, the baseline HR remained normal.

When compared to the control rats, the tachycardiac response induced by methylatropine treatment was reduced in the BDNF-treated rats, while the bradycardiac response induced by propranolol was enhanced. Cardiac sympathetic influence was enhanced and parasympathetic influence was attenuated in the BDNF-treated rats (Table 1).

Fig. 1 shows an overall view of the MAP-HR relationship plotted by the averaged HR changes versus the averaged MAP changes in response to the various doses of PE or NP. Statistics of individual BRS data are also shown (Fig. 1, d and e). The BRS in reflex bradycardia during PE-induced pressor responses (PE-BRS) was significantly enhanced (more negative) in the BDNF-treated rats (Fig. 1, a and d), whereas the BRS in reflex tachycardia during NP-induced depressor responses (NP-BRS) was not changed (Fig. 1, a and e).

The involvements of sympathetic and parasympathetic components in BRS were further determined after methylatropine (a pharmacologic parasympathetic blocking agent) or propranolol (a pharmacologic sympathetic blocking agent) treatment, respectively (Fig. 1, b and c). After blockade of the parasympathetic nerve with methylatropine, the PE-BRS in the BDNF-treated rats was the same as that in the control rats. However, the PE-BRS remained enhanced after sympathetic blockade with propranolol (Fig. 1d). For the NP-BRS, there was no difference between both groups before or after methylatropine or propranolol treatments (Fig. 1e).

Table 2 shows the MCRi (the metabolic clearance rate of insulin), GIR (glucose infusion rate), and Si (an index of whole-body insulin sensitivity) in the steady-state period of the euglycemic hyperinsulinemic clamp study. In the basal period, BDNF-treated rats showed significantly lower fasting blood glucose and fasting plasma insulin levels. During the steady-state period (clamp $_{90\text{-}150\text{-min}}$) of the euglycemic hyperinsulinemic clamp study, blood glucose in all rats was maintained around a euglycemic level by exogenous glucose infusion. The plasma insulin levels and MCRi in the BDNF-treated rats were not significantly different from those of the control rats. However, GIR and Si were increased in the BDNF-treated rats (Table 2).

Discussion

The present study demonstrated that intracerebroventricular administration of BDNF in rats could elevate blood pressure, enhance the sensitivity of reflex bradycardia (PE-BRS), and increase insulin sensitivity (Si). In addition, the enhanced PE-BRS observed in the BDNF-treated rats was likely due to the abnormal parasympathetic activity.

In the present study, intracerebroventricular administration of BDNF resulted in enhancement of PE-BRS (Fig. 1). Several lines of evidence have suggested that BDNF is involved in the development and physiological activity of the arterial baroreceptor

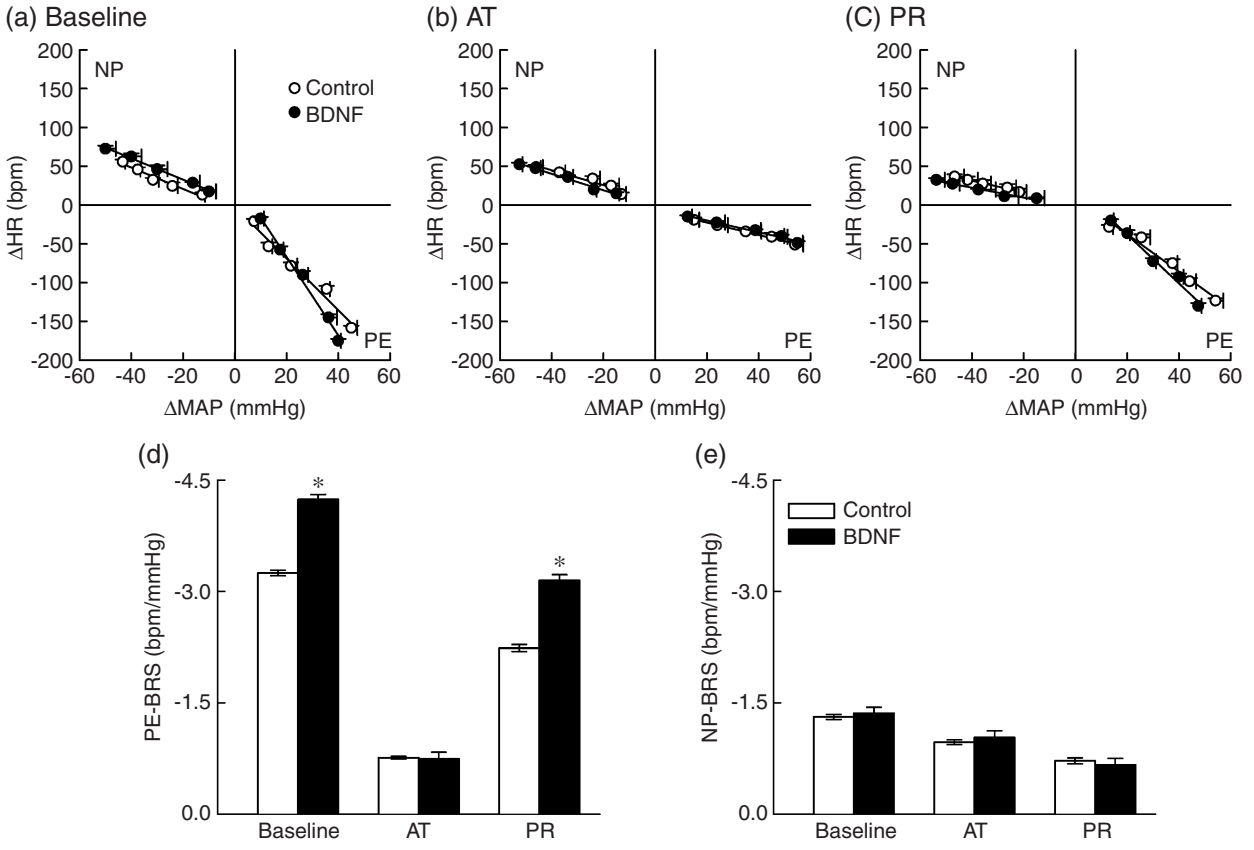

Fig. 1. Baroreflex sensitivity after intracerebroventricular administration of BDNF in rats. The regression lines (top panel) represent the overall view of the MAP-HR relationship at baseline (a), after methylatropine (AT; b), or after propranolol (PR; c) treatment in rats after intracerebroventricular administration of BDNF (BDNF; n = 10) or artificial cerebrospinal fluid (control; n = 10). The regression lines for overall view were calculated from the averaged heart rate changes (ΔHR) versus the averaged arterial blood pressure changes (ΔMAP) in response to various doses of phenylephrine (PE) or sodium nitroprusside (NP) in the BDNF-treated and control rats. The column charts (lower panel), however, illustrate the statistics of individual PE-BRS (d) and NP-BRS (e) data, of which the regression lines were constructed from individual but not averaged data points, from the BDNF-treated and control rats. *$P < 0.05$ *vs*. the corresponding value of the control group. Values are expressed as means ± SEM.

Table 2. Blood glucose, plasma insulin, MCRi, GIR, and Si values during euglycemic hyperinsulinemic clamp experiment after intracerebroventricular administration of BDNF in rats

	Control (n = 8)	BDNF (n = 8)
Basal period		
Fasting blood glucose (mM)	5.3 ± 0.2	4.9 ± 0.1*
Fasting plasma insulin (pM)	162 ± 8	98 ± 7*
Clamp $_{90\text{-}150\ min}$ period		
Blood glucose (mM)	5.4 ± 0.2	5.3 ± 0.3
Plasma insulin (pM)	886 ± 29	876 ± 28
GIR (mg/kg · min)	16 ± 1	26 ± 2*
MCRi (ml/kg · min)	33 ± 1	32 ± 2
Si (l/kg · min)	99 ± 5	169 ± 12*

Clamp $_{90\text{-}150\ min}$, the steady-state of euglycemic hyperinsulinemic clamp experiment; GIR, glucose infusion rate; MCRi, metabolic clearance rate of insulin; Si, index of whole-body insulin sensitivity, calculated by the ratio of GIR to plasma insulin levels during Clamp $_{90\text{-}150\ min}$ period; Control, rats received intracerebroventricular injection of artificial cerebrospinal fluid; BDNF, rats received intracerebroventricular injection of BDNF. *$P < 0.05$ compare to the corresponding values of the control group. Values are expressed as means ± SEM.

system (3, 21). BDNF and trkB receptors, the high-affinity receptor of BDNF, could be found in the lower brain stem (5), and were also abundantly expressed in the hypothalamus (6, 36) which has been shown to play a critical role in the regulation of the autonomic nervous system and energy homeostasis. Several studies demonstrated that intracerebroventricular administration of BDNF in doses as small as 0.2-5 μg/rat (13, 26, 35) or 1.5-15 μg/mouse (25, 32) had significant effects on the metabolic, cardiovascular, and stress responses of the animals, suggesting that intracerebroventricular BDNF could gain access to the hypothalamic area. Neurons of hypothalamus send projections to the lower brain stem to integrate the baroreflex circuits (4, 8). BDNF could act as neurotransmitters (33) to modulate synaptic transmission and regulate other neurotransmitters, such as GABA (2), glutamate (1), and serotonin (20). Co-existence of BDNF with catecholaminergic neurons in the lower brain stem of rats also implicates the involvement of BDNF in central cardiovascular regulation (5). Moreover, activation of trkB receptors was shown to be responsible for BDNF-mediated synaptic plastic changes in the baroafferent sensory relay neurons of the nucleus tractus solitarii (NTS), the first central target of baroreceptor afferent in the lower brain stem (1). Neurons in the rostral ventrolateral medulla, a central cardiovascular regulatory center containing neurons which send projections to preganglionic sympathetic neurons, could also be activated by BDNF and elicit increases of blood pressure in rats (33). These observations are consistent with our findings and clearly indicate that brain BDNF may have a physiological role in the regulation of the cardiovascular reflex function.

Our results demonstrated the contributory role of abnormal parasympathetic activity in the abnormal PE-BRS in the BDNF-treated rats, since blockade with methylatropine but not propranolol, was able to normalize the PE-BRS (Fig. 1d). The cardiac parasympathetic influence, which represented the resting parasympathetic tone, was decreased after BDNF treatment (Table 1). The lower resting parasympathetic activity might be more readily activated in response to hypertensive challenge (PE). Therefore, the PE-BRS was enhanced in the BDNF-treated rats.

Both *in vitro* and *in vivo* studies demonstrated that BDNF has a direct action on enhanced sympathetic outflow (33, 37). Studies in central control of parasympathetic outflow also showed retrograde transport of BDNF in the regulation of cholinergic outflow (14, 38). In addition, in the BDNF heterozygous knockout (BDNF+/-) mice, the resting HR was elevated, suggesting a role of endogenous BDNF in cardiac autonomic regulation (23). In the present study, the baseline HR in BDNF-treated rats remained intact, although cardiac sympathetic influence was increased and cardiac parasympathetic influence was decreased (Table 1). The intact baseline HR might have resulted from the interaction between the enhanced PE-BRS (promoting reflex bradycardia) and the enhanced cardiac sympathetic influences. Our results also showed a slight but significant increase in blood pressure after central BDNF treatment (Table 1), consistent with the results of another study (26). Taken together, these findings suggest that central BDNF may play an important role in the regulation of cardiovascular sympathetic and parasympathetic functions.

In addition to the regulation of cardiovascular autonomic function, intracerebroventricular administration of BDNF also enhanced glucose disposal (GIR) and insulin sensitivity (Si), as revealed in the clamp study (Table 2). BDNF has been indicated to be involved in the regulation of a variety of physiological variables and activities, such as glucose metabolism, obesity, and energy expenditure, due to its action on the hypothalamic neurons (9, 28, 32). A single intracerebroventricular injection of BDNF in obese diabetic mice could rapidly activate norepinephrine turnover, enhance thermogenesis, and stimulate expression of uncoupling protein 1 gene in brown adipose tissue (28). This indicated that central BDNF activated sympathetic outflow was able to regulate energy expenditure. Sympathetic nervous system is known to be responsible for modulation of both hepatic glucose production and glucose uptake in peripheral tissues (27). Acute activation in sympathetic outflow by central stimulation, leading to focal norepinephrine release from sympathetic nervous terminal, was shown to predominately contribute to the increases of glucose uptake in peripheral tissues (19, 24). It was consistent with our results that the increases in glucose disposal and insulin sensitivity were accompanied by the augmented sympathetic activity after the single intracerebroventricular injection of BDNF. These sympathetic-related increases in glucose disposal and insulin sensitivity might also account for the slightly lowered blood glucose levels, which, however, still remained within the normal physiological range (Table 1 and 2). It was reported that chronic and repetitive subcutaneous administration of BDNF (20 mg/kg per day) had no effects on the blood glucose levels in normal mice (29). The discrepancy between ours and that study might be due to differences in the species and the dosage used, and the duration and route of BDNF administration. On the other hand, stimulation of parasympathetic outflow, conveying signals from the hypothalamus to the adipose tissues and pancreatic β cells (7), was reported able to directly enhance insulin secretion (12) and increase pancreatic β cell mass (10, 17). Thus, with a normal metabolic clearance rate of in-

sulin (MCRi), the lowering of plasma insulin levels might attribute to the attenuated parasympathetic activity in the BDNF-treated rats. Similar to the effects of central BDNF on PE-BRS and HR as previously described, our results indicate that effects of central BDNF on the glucose metabolism and insulin sensitivity might be due to action of BDNF on the modulation of autonomic nerve activities.

In conclusion, the results of the present study demonstrated that intracerebroventricular BDNF could rapidly enhance insulin sensitivity and BRS. These acute central BDNF effects might work through modulation of the autonomic nervous system. Autonomic dysfunction and metabolic impairment are frequently found to be associated with lower plasma BDNF levels in patients with diabetes (18, 23) as well as with dementia and depression (22). Therefore, the role of BDNF on regulation of cardiovascular autonomic function and insulin sensitivity reported in the present study may be of potential clinical therapeutic importance.

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